

## Evaluation of the action of several antifungal agents in *Candida* species biofilms

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### Abstract

*Candida* species are currently one of the most common causes of nosocomial infections, with a growing incidence of non-*Candida albicans* *Candida* (NCAC) species. Therefore, it is of utmost importance to assess their virulence, specifically biofilm formation ability and resistance to antifungal agents. Different mechanisms can be responsible for the known intrinsic resistance of biofilms cells to antimicrobial agents. These include effects of the biofilm matrix on drug penetration, decreased cell growth rate, nutrient limitation, expression of drug-resistance genes (particularly those encoding efflux pumps) and the presence of “persister” cells. The interaction of *Candida albicans* biofilms with antimicrobial agents have been thoroughly evaluated, however little is known about the virulence of NCAC biofilms. Hence, the goal of this work was to determine the resistance of several NCAC species, specifically *C. albicans*, *C. tropicalis*, *C. parapsilosis* and *C. glabrata*, in biofilm form in comparison with planktonic cells against the most common azole antifungal agents.

Accordingly, the action of fluconazole and itraconazole was evaluated on one reference strain of *C. albicans* and one clinical isolate of *C. tropicalis*, *C. glabrata* and *C. parapsilosis*. Antifungal susceptibility was evaluated by the microdilution method, as described by CLSI M27-A2. Additionally, the ability of NCAC species to form biofilms in the presence of antifungals and the effect of these drugs in mature biofilms (24 h) was assessed by the Crystal Violet staining.

The results obtained demonstrated that the most sensitive species to both antifungal agents was *C. tropicalis*, when evaluated according to the standard, but when crystal violet staining was used to assess biofilm biomass, *C. parapsilosis* showed to be the most sensitive one. In opposition, *C. glabrata* was the strain that presented the highest resistance to both antifungal agents. A very low degree of action of the antifungal agents in the pre-formed biofilms was also observed, as well as the occasional occurrence of a slight stimulation of biofilm formation.

Thus, it is possible to conclude that *Candida* biofilms demonstrate higher antifungal resistance rates than planktonic cells and also that the antifungal agents tested show a very low degree of action on pre-formed biofilms.